PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P.ULB.81/WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/BE 03/00147	International filing date *(day/month/year)* 03.09.2003	Priority date *(day/month/year)* 03.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/10		
Applicant UNIVERSITE LIBRE DE BRUXELLES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.02.2004	23.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Hornig, H Telephone No. +31 70 340-2620

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE 03/00147

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-13 as originally filed

Claims, Numbers

1-21 received on 07.09.2004 with letter of 07.09.2004

Drawings, Sheets

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 13-18

   because:

   ☒ the said international application, or the said claims Nos. 13-18 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,3,13-21
	No:	Claims	1,4-12
Inventive step (IS)	Yes:	Claims	2,3,13-21
	No:	Claims	1,4-12
Industrial applicability (IA)	Yes:	Claims	1-12,19-21
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

1.1 The amended claims 1-21 filed with the letter dated 07.09.2004 and received 07.09.2004 are allowable according to Art. 34(2)(b) PCT. The basis of the report issues on the claims 1-21 as amended according to Rule 70.2 PCT.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1.1 Claims 13-18 relate to subject-matter considered by this Authority to be covered by the provision of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

1.1 After reconsidering the application and although the claims have been drafted as being dependent on claim 1, they are clearly defined as **three** different independent subject-matters and have to be subdivided into three different inventions. The reasons for the objection being as follows:

(I) Alternative methods for the selection of recombinant clones having integrated a gene of interest (6) and a nucleotide sequence (3) encoding a functional antidote protein (4) to a toxic molecule (5), wherein said recombinant clones are the ones which survive following their integration into a host cell (20) comprising in its genome a nucleotide sequence (21) encoding said toxic molecule (5) **(D1),** and
(ii) alternative double selection vector (1) able to transform a cell of a specific organism which contains two different genes (2 and 3) comprising several cloning sites and encoding two different toxic molecules (4 and 5) to a prokaryote cell, said genes being disposed in opposite lecture orientation to each other, upstream and downstream a cassette sequence (6) and is also related to a method for the genetic modification of a cell or an organism comprising the use of said double selection vector **(D2)**, are already state of the art **(D1, D2)**.

In the light of the prior art the problem addressed by the applicant is the provision of alternative genetic constructs for an insertion, deletion and/or inversion of a target nucleotide sequence (A) into a cell.

Due to the fact that alternative methods for the selection of recombinant clones encoding a functional antidote protein are known, and alternative double selection vector encoding two different toxic molecules, able to transform a cell and methods for the genetic modification of a cell or an organism comprising the use of said double selection vector are already state of the art, the examining Division is of the opinion that there is **no** single inventive concept underlying the present application.

Due to the essential differences of the constructs respectively the provided 3 different solutions and due to the fact that no other technical features can be distinguished which, in the light of the prior art, could be regarded as special technical features common to these solutions, there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is lack of unity and the **three** different inventions, not belonging to a common inventive concept, are formulated as follows:

(1) Claims (1,4-21)-partially; A genetic construct (10) which is suitable for the insertion/ deletion and inversion for at least one target nucleotide sequence (A) and comprised of: (I) - a promoter/ activator sequence (11) disposed upstream of a first and a second nucleotide sequence (1, 2) encoding two different toxic molecules;

(2) Claims (1,4-21)-partially; A genetic construct (10) which is suitable for the insertion/deletion and inversion for at least one target nucleotide sequence (A) and comprised of: (ii) - a first promoter/ activator sequence (11) disposed upstream of a first nucleotide sequence (1) encoding a toxic molecule and disposed in the opposite direction, a second promoter/ activator sequence (12) disposed upstream of a second nucleotide sequence encoding an antidote (2') to a second toxic molecule different from said first one;

(3) Claims (1-21)-partially; A genetic construct (10) which is suitable for the insertion/deletion and inversion for at least one target nucleotide sequence (A) and comprised of: (iii) - a promoter/ activator sequence (11) disposed upstream of a first nucleotide sequence (1) encoding a first toxic molecule and a second nucleotide sequence (2') encoding an antidote to a second toxic molecule different from said first toxic molecule and, disposed in the opposite direction to the lecture orientation of the

first promoter/activator sequence (11); said genetic construct wherein the third nucleotide sequence (1') encoding an antidote to the first toxic molecule is under the control of a second promoter/activator sequence (12);

1.2 The international examination Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees, because all claims could be examined without extra effort.

1.2.1 Nevertheless, should the application enters the regional phase, the examining division is free to decide again on the existence of unity of the invention, respectively to invite the applicant to restrict or to pay additional examination fees.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 02 066657 A (SZPIRER CEDRIC YVES ;VAN MELDEREN LAURENCE (BE); GABANT PHILIPPE () 29 August 2002 (2002-08-29) cited in the application

D2: EP 1 111 061 A (UNIV BRUXELLES) 27 June 2001 (2001-06-27)

2. Novelty (Article 33(2) PCT)

2.1 D1 relates to a method for the selection of recombinant clones having integrated a gene of interest (6) and a nucleotide sequence (3) encoding a functional antidote protein (4) to a toxic molecule (5), wherein said recombinant clones are the ones which survive following their integration into a host cell (20) comprising in its genome a nucleotide sequence (21) encoding said toxic molecule (5).

Furthermore D1 describes in Fig. 1 a nucleic construct (1) comprising a nucleic acid sequence (2) made of a nucleic acid sequence 3, encoding an antidote protein 4 **(CcdA protein)** to a toxic molecule (5) **(CcdB protein)** and a gene of interest (6), and a

promoter/operator sequence (9); said cassette sequence (2) being disposed between a first recombination site (7) and a second recombination site (8), which do not recombine with each other. Said nucleic acid construct (1) is integrated into a further vector which is an insert donor DNA vector (10) comprising a further selectable marker (11) **(Kid protein)**. The insert donor DNA vector 10 could be amplified in a bacteria which is resistant to the activity of the first selectable marker 11 (kid protein), for instance, a bacteria expressing the antidote protein kis to the protein poison kid. This means that the endogenous **kid promoter** must be active in said bacteria.

The wording of claim 1: **"A genetic construct (10)... comprised of: (iii)... a promoter/ activator sequence** (kid promoter in D1) **(11) disposed upstream of a first nucleotide sequence (1) encoding a first toxic molecule** (Kid protein in D1) **and a second nucleotide sequence (2') encoding an antidote to a second toxic molecule** (CcdA protein in D1) **different from said first toxic molecule",** falls into the scope of Fig. 1 in D1.

2.1.1 Therefore the subject-matter of claims **1(iii)** and **(4-12)**-partially does not meet the requirement of Article 33(2) and (3) PCT.

3. Inventive step (Article 33(3) PCT)

3.1 D1 relates to a method for the selection of recombinant clones having integrated a gene of interest (6) and a nucleotide sequence (3) encoding a functional antidote protein (4) to a toxic molecule (5), wherein said recombinant clones are the ones which survive following their integration into a host cell (20) comprising in its genome a nucleotide sequence (21) encoding said toxic molecule (5).

D1, regarded as the closest state of the art, differs from the subject-matter of claim **1(ii)** that it does not describe genetic constructs encoding a first nucleotide sequence encoding an toxic molecule and disposed **in opposite** direction to a second nucleotide sequence encoding an antidote to a second toxic molecule. In the light of the prior art, the problem addressed by the applicant is the provision of alternative genetic constructs for an insertion, deletion and/or inversion of a target nucleotide sequence (A) into a cell. The solution as provided by the applicant is:

"A genetic construct (10)... comprised of:
(ii) - a first promoter/ activator sequence (11) disposed upstream of a first

nucleotide sequence (1) encoding a toxic molecule and disposed in the opposite direction, a second promoter/ activator sequence (12) disposed upstream of a second nucleotide sequence encoding an antidote (2') to a second toxic molecule different from said first one;

3.1.1 The technical feature of the independent claim **1(ii)** is neither known from, nor rendered obvious by, the available prior art. In the light of D1 the subject-matter of claim 1(ii) does meet the requirement of Article 33(3) PCT.

3.2 D2 describes a double selection vector (1) able to transform a cell of a specific organism which contains two different genes (2 and 3) comprising several cloning sites and encoding two different toxic molecules (4 and 5) to a prokaryote cell, said genes being disposed in opposite lecture orientation to each other, upstream and downstream a cassette sequence (6) and is also related to a method for the genetic modification of a cell or an organism comprising the use of said double selection vector.

D2, regarded as the closest state of the art, differs from the subject-matter of claim **1(I)** that it does describe a double selection vector comprising a genetic constructs which contains two different genes encoding two different toxic molecules, and wherein said genes being disposed in **opposite** lecture orientation to each other. n the light of the prior art, the problem addressed by the applicant is the provision of alternative genetic constructs for an insertion, deletion and/or inversion of a target nucleotide sequence (A) into a cell. The solution as provided by the applicant is:

"A genetic construct (10)... comprised of either:
(I) - a promoter/ activator sequence (11) disposed upstream of a first and a second nucleotide sequence (1, 2) encoding two different toxic molecules".

In the light of D2, it is not obvious for a man skilled in the art, that a selection vector comprising a genetic constructs which contains a promoter/ activator sequence disposed upstream of two different genes encoding two different toxic molecules, and wherein said genes being disposed in **direct** lecture orientation to each other.

3.2.1 In the light of D2 the subject-matter of claim 1(I) does meet the requirement of Article 33(3) PCT.

4. Further comments

4.1 Claim 3, dependent on claim 2, is unclear under Article 6 PCT. Claim 3 reffers to a **third** nucleotide sequence (1') which is not disclosed respectively mentioned in claim 2, thereby rendering claim 3 unclear. Furthermore the orientation of the second promoter/activator sequence (12) in respect to the first promoter/activator sequence (11) is unclear.

4.2 In claims 9 and 13 the expression "preferably" has been considered as having no limiting effect on the scope of said claim (see EPC Guidelines III-4.6 read in connection of Article 6 PCT).

4.3 The same applies to claims 13,14 and 21, the features following the adverb "possibly" have been regarded as entirely optional and therefore as features having no limiting effect on the scope of said claim (see EPC Guidelines III-4.6 in connection of Article 6 PCT).

4.4 It is not clear what is meant with the expression " **(11°)** " in claim 2, rendering the scope of said claim unclear (Article 6 PCT). It could be an clerical error and should be read as **(11)**.

4.5 The expression "..encoding two different **toxic** molecules" is vague and not clear under Art. 6 PCT. In principle any protein can be "toxic" to a host cell from a certain concentration upwards.

4.6 The present claims 19 and 20 are not clear. The term: **"computer program comprising program codes means for performing the steps .."** is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Art. 6 PCT).

4.7 The present claim 21, "**An automate** connected to a database of a computer and which comprises ...the **computer program comprising program codes means for** performing the method of claims 13 to 18" is not clear (Art. 6 PCT), in that the matter for which protection is sought is not defined. Furthermore claim 21 contain **a mixture of categories of claims.** The claim attempts to define the subject-matter (an automate) in terms of the result to be achieved (..comprising program code means for performing the method of ..).